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Some Aspects About Using *Bacterial Pure Cultures* in the Manufacture of Fermented Sausages

■ USING MICROBES to improve the flavor and keeping quality of foods is as old as the art of food preparation. Not until the time of Pasteur, however, was it realized that microbes are responsible for processes such as the fermentation of beer and wine, the souring of bread dough, and the ripening of cheese. Soon after this observation, people also learned to cultivate these microbes as pure cultures and were thus able to both activate and control the desired fermentations.

At present the development in some fields of food manufacture has already got so far—thanks to intensive research work—that bacterial fermentations have been made quite automatic. An example of this is the use of suitable pure cultures for souring cream in modern dairies.

The reasons why investigations of the use of bacterial pure cultures in the manufacture of fermented meat products are still in their beginnings might be found in following facts:

1) Research in meat technology has, in most countries, been left behind research in, e.g., milk technology. 2) Meat as research material is much more difficult to examine than milk or dairy products, because of its great inhomogeneity. 3) Investigations into the effects of bacterial pure cultures present difficulties because it is not possible to experiment with sterile or pasteurized material, as can be done in the manufacture of dairy products. The effects of undesired random bacteria in meat products cannot be eliminated. The number of such undesired bacteria can be several millions per gram, and their influence on chemical changes is more significant than the influence of inoculated bacteria.

Efforts to bring bacterial pure cultures into the scope of meat manufacture have not, however, been lacking. The first of these efforts originated from the USA (Drake, 1928; Jensen and Paddock, 1940; Kurk, 1921). In 1955 both Niven *et al.* (1955) and Niinivaara (1955) presented new conceptions of the use of bacterial pure cultures in the manufacture of fermented meat products. As a suitable strain for fermented sausage common in the USA, Niven (1955) suggested the strain *Pediococcus cerevisiae,* and Niinivaara (1955) for the European types of fermented sausage ("Rohwurst") *Micrococcus* strain M53 or, in general, suitable strains of the genus *Micrococcus* ("Bergey's Manual," 6th ed.). Since around 1957, both of these bacterial cultures have been available as lyophilized commercial products for use in manufacturing fermented sausages, the *Pediococcus* under the name "Accel," produced by Merck & Co., Rahway, N. J., USA, and the *Micrococcus* under the name "Baktofermente," produced by Rudolf Müller Co., Hamburg, Germany.

75 g glucose
12 g nitrate
87 g white pepper
5 g cardamon

All meats are frozen and ground in that state. After being sprayed, the sausages are predried for 3–5 days in air-conditioned chambers (Örico or Autotherm) and then smoked for about 5–7 days. During drying and smoking, the temperature was 20°C. After being smoked, the sausages were stored about 10 days at 10°C.

Some Microbiological Aspects

We were experimenting with various bacteria cultures isolated from fermented meat products or curing brines to determine their suitability for the manufacture of dry sausage. We soon observed that it was not possible to obtain any results without having a suitable *selection procedure* for this purpose. A selection procedure (Fig. 1) suitable for the testing of micrococci was then developed (Pohja, 1960). The grouping scheme of this selection procedure includes the investigations of 4 characteristics: 1) ability to reduce nitrate; 2) ability to form acid from glucose in aerobic conditions; 3) ability to grow in media containing NaCl; and 4) intensity of nitrate reduction.

According to this selection procedure, the ability to reduce nitrate is tested first. Only the strains with positive reaction will be tested further, and their ability to produce acid from glucose is then tested. Rate of growth is observed because only vigorously growing strains can be used. Lastly, the intensity of nitrate reduction is tested.

Of more than 700 strains tested with this procedure, only 3 were useful for dry sausage manufacture. Rather few strains among micrococci seem to be suitable for this purpose.

Experience has proved the same strain cannot be used a very long time in the cultures because, in time, it loses part of its desired characteristics. This was the case with, for example, the strain *Micrococcus* M53, which in 1953 was found especially suitable for this purpose but now is completely degenerated. It is therefore necessary to select *continuously* new strains from succesful fermented meat products or curing brines.

The before-mentioned scheme has made the investigation and selection of new *Micrococcus* strains very much easier. The grouping scheme can, of course, be formed also in other ways, and perhaps be made more complete and accurate after it can be shown which characteristics other than those previously mentioned are essential for the strains suitable for use in the manufacture of dry sausage.

Certain difficulties are connected with cultivating bacteria on a large scale. Niven (1961) showed that the salt tolerance of lyophilized bacteria decreases. In the cultivation of our micrococci, some strains showed a great sensitivity to phages (Gyllenberg, 1962); several cultures were completely destroyed (Fig. 2).

Changes in bacterial flora during the fermentation period of dry sausage were rather thoroughly examined in our previous investigations (Niinivaara and Pohja, 1956 and 1957; Pohja and Niinivaara, 1957). The present studies confirmed the earlier observations. The NaCl content of the sausage is increased by drying, and this fact obviously influences the bacterial flora. This is very distinctly shown in examination of the bacteria that proteolyze meat (Pohja *et al.*, 1960).

The correlation between bacteria content and NaCl content becomes more evident when the NaCl content of the *water phase,* instead of total NaCl content, is used as the comparison basis (Fig. 3).

Some Chemical Aspects

Earlier investigations of the influence of different bacteria cultures on

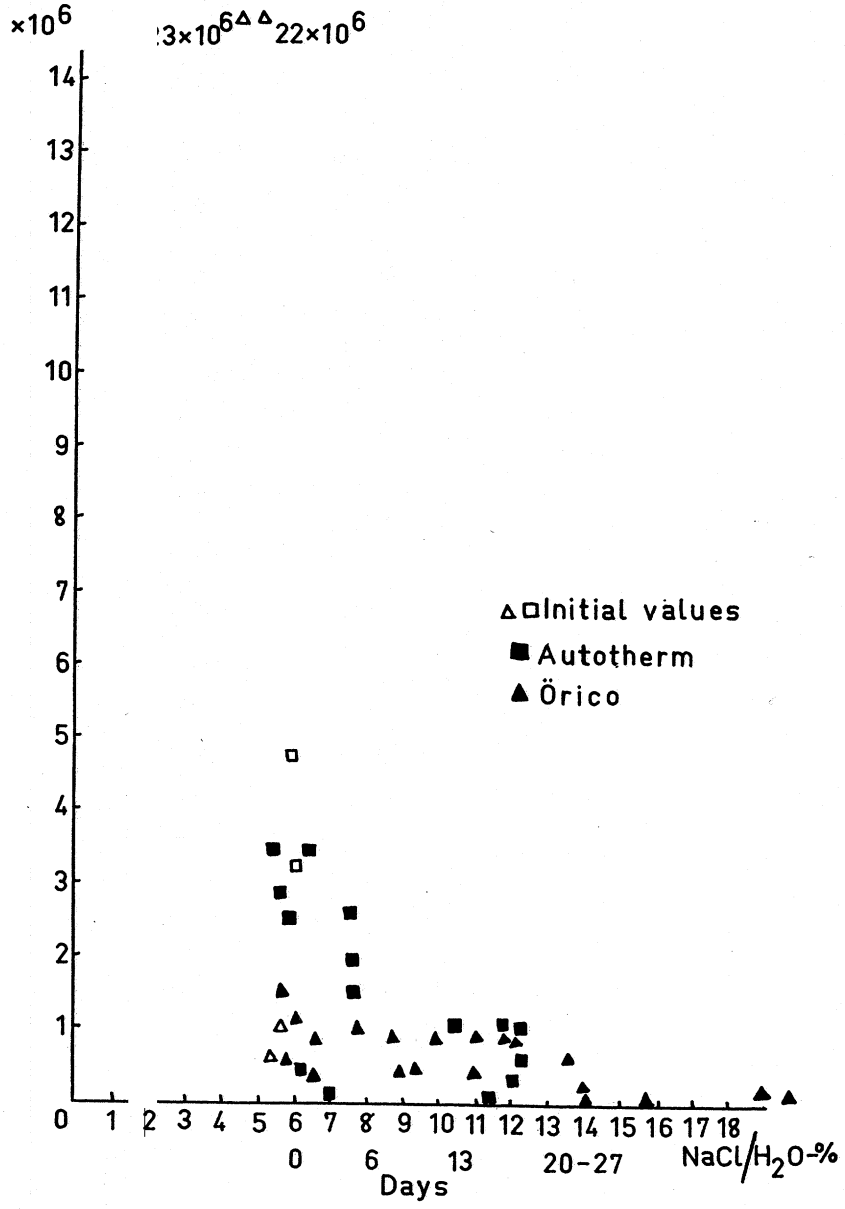

Fig. 3. Meat-proteolyzing bacteria in dry sausage during 27 days fermentation, correlation to NaCl/H_2O conc.

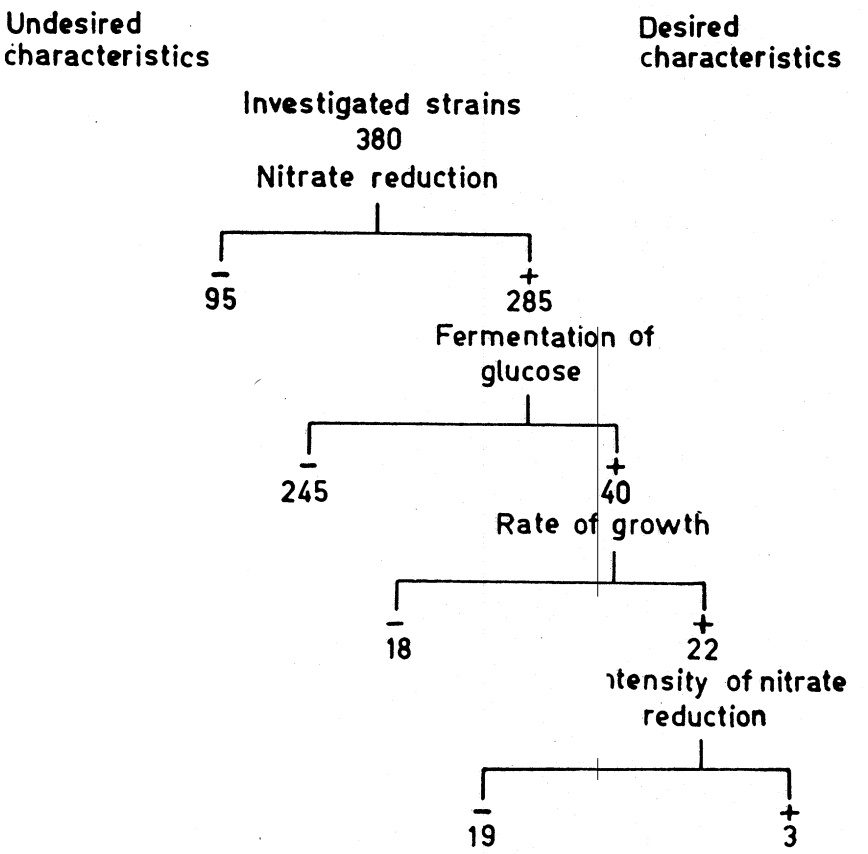

Fig. 1. Diagram showing the selection procedu for *Micrococcus* strains suitable for use in the manufacture of dry sausages.

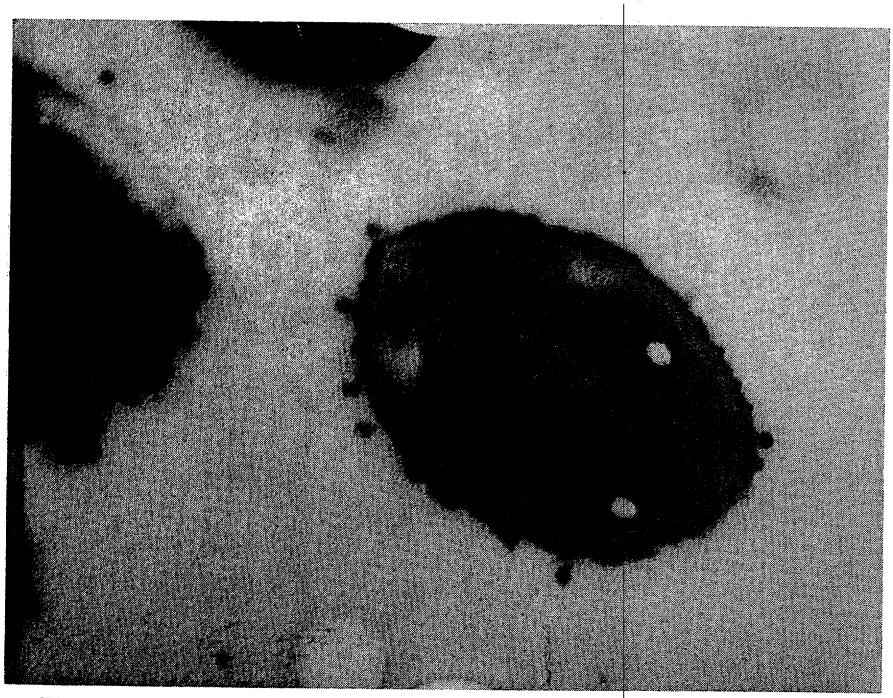

Fig. 2. Micrococcus M53 (94000 ×) infected by phages. (Courtesy of Gyllenberg, 1962.)

When our knowledge has increased and we know what chemical changes desired flavor, texture, and color are based on, and which bacteria are responsible for each of these changes, we most certainly will get better results by inoculating the same product simultaneously with pure cultures or mixed cultures of several bacteria.

European Types of Fermented Sausages

When comparisons are made between several investigations in dry sausages, great difficulties always result from the fact that dry sausages can be widely different from each other in method of production and chemical composition. The processing time of European dry sausages varies from 10 to 100 days and even more. The Hungarian salami, for example, can be 6 months old when it goes on sale. Some dry sausages are smoked; others are not. On the surface of unsmoked sausages, a strong mold growth is usually allowed to develop. This mold gives a very special flavor to the sausage.

In some countries dry sausage is prepared by soaking it in brine. Water diffuses from the sausage into the brine, and salt from the brine into the sausage. After this "drying" period, the sausage is smoked. In this way the sausage usually becomes very salty, and its fine meat flavor is often concealed by the taste of salt. It looks, however, as if this method would everywhere be giving way to the method of drying in air.

Additional development along the same line is the more common use of the automatic drying equipment (e.g., Autotherm, which at present has been used in all our investigations). Also, the fact that the starter cultures are now being adopted has improved the manufacturing process of dry sausage, the danger of the failure now becoming insignificant.

In our investigations, dry sausage has always been prepared in the following way:

Ingredients:

35 kg beef (shoulder, rib, and loin; round about 60%)
35 kg pork (shoulder, loin, ham)
30 kg pork fat
40 g spice mixture
50 g Baktofermente, pure culture of Micrococci
350 cc red wine
(100 g phosphate mixture "Fibrisol 424")

Spice mixture:

821 g salt

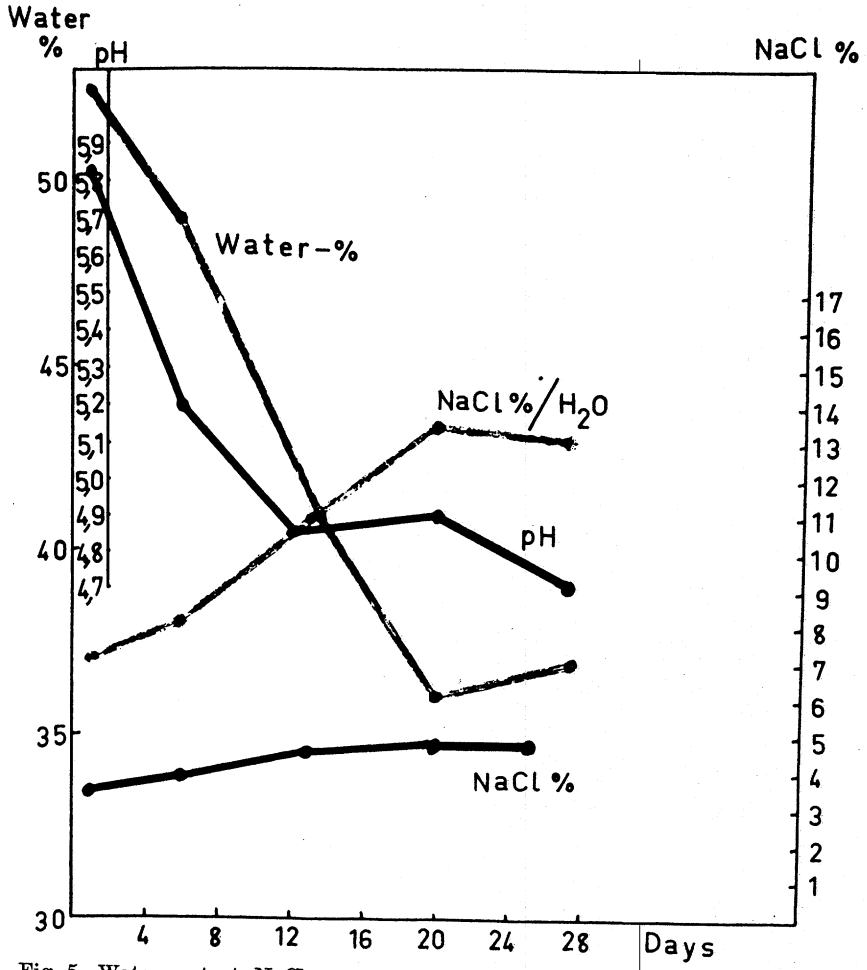

Fig. 5. Water content, NaCl concentration and pH value during 27 days fermentation.

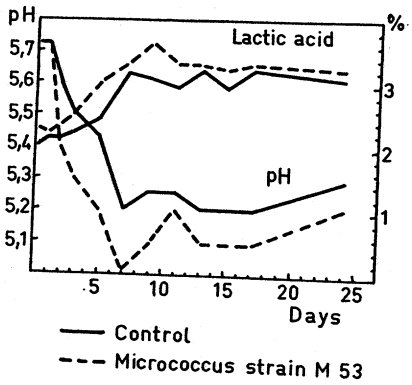

Fig. 6. Production of lactic acid.

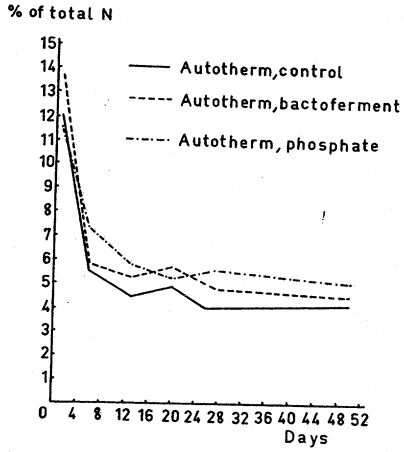

Fig. 7. Soluble protein N.

cant. Inoculation had no effect on the changes in the amino acids. It looks as if the tissue enzymes, instead of bacteria, would be responsible for the proteolytic changes of the proteins during the first phase of ripening (*cf.* De Silva and Hughes, 1962).

Fats and Carbonyls. In our investigations until now it has not been possible to take special notice of the changes in the fat fraction. Obviously, however, just these compounds are of great significance in the flavor of dry sausage. This will be discussed more closely in the following section.

For the present we have examined only the carbonyl fraction extracted from dry sausage. In preparing the sample for the gas chromatographic studies the carbonyls were isolated as 2,4-dinitrophenylhydrazones, regenerated with α-ketoglutaric acid and immediately analyzed. In this fraction, 30–40 different compounds could be shown by gas chromatography (Fig. 10). In our opinion the carbonyl fraction plays a significant part in the flavor of dry sausage, at least in sausages that are older and produced with a long ripening period. The developing of this method would open new possibilities to examine the correlation between the bacteria and the flavor of dry sausage. The subjective methods used up to now (organoleptic evaluation) are insufficient for the purpose of getting an accurate picture of the part that bacteria play in the chemical changes.

On the basis of preliminary studies it has already been possible to show

New Possibilities

Our insight into the chemistry of the flavor of meat is still rather limited, and that on meat products, including dry sausage, is almost nonexistent. We recently began research on the chemical compounds that produce the characteristic flavor of dry sausage. *Gas chromatographic methods* seem very well fitted for this purpose.

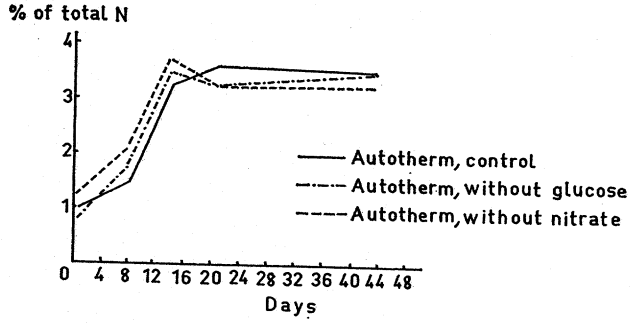

Fig. 8. Amino N, water extract.

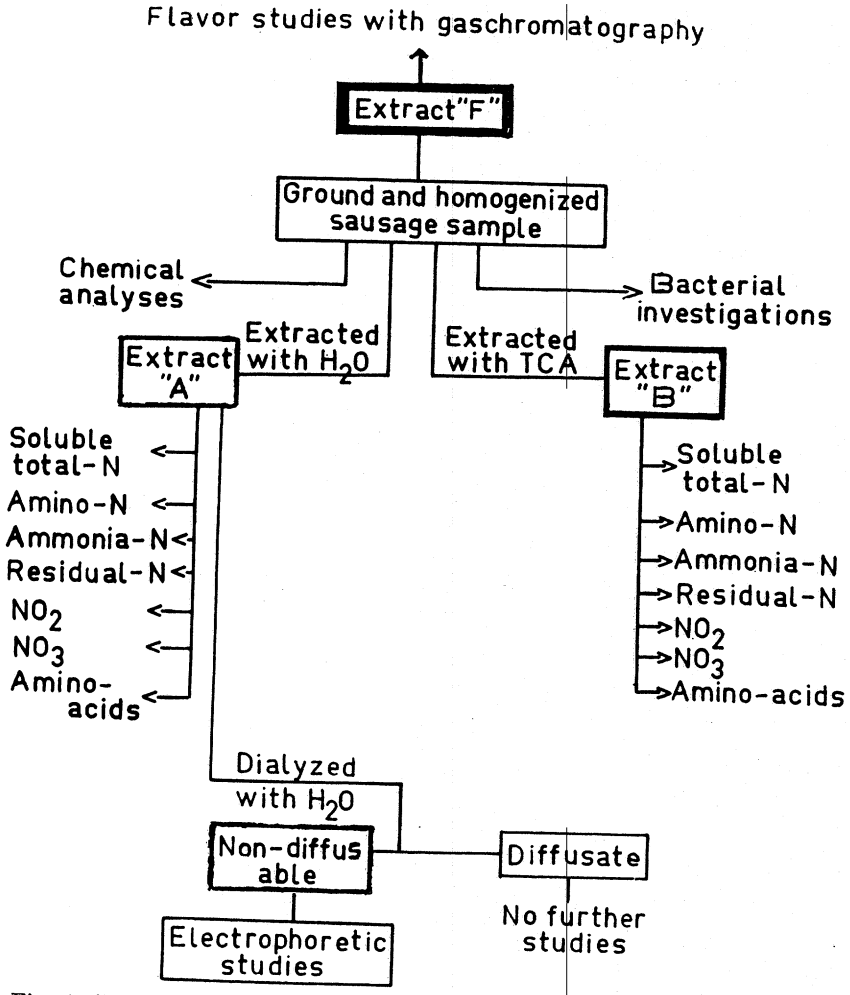

Fig. 4. Procedure used for fractionation of dry sausage during fermentation.

the characteristics of dry sausage made use of organoleptic evaluation (e.g., Keller, 1953). The *correlation between certain bacteria and chemical changes* during fermentation has not been investigated yet. We have, therefore, made some efforts to solve this question.

The aim of the study was to prove the effects of certain bacteria cultures, added chemicals (NO_3, glucose), and processing conditions upon chemical changes, especially those changes that presumably influence the progress of ripening. Fig. 4 shows the progress of this study. The following determinations were made at regular intervals during ripening:

1) The chemical composition of the sausage (water, fat, protein, salt).

2) The total number of bacteria, the number of anaerobic and proteolytic bacteria.

3) The water extract was analyzed, among others, for soluble total nitrogen, residue nitrogen, NO_2, NO_3, the presence of free amino acids and their amount (half quantitatively).

4) Trichloroacetic acid (TCA) extract was analyzed for the same things as in section 3.

5) The water extract was dialyzed against water, and the non-diffusable protein was fractionated electrophoretically.

6) An extract was prepared from dry sausage, marked "F," in order to investigate the chemical composition of its flavor.

The chemical composition of dry sausage is changed during the ripening process because of the evaporation of water (Fig. 5). These changes (principally the increase of NaCl content) affect the composition of the bacterial population of dry sausage.

The inoculation cannot be shown to have any effect on the changes in chemical composition (Figs. 4, 5). The rate of drying depends on the original composition of the sausage and the drying conditions. During the drying, however, changes take place not only in the amount but also in the chemical composition of the organic compounds of the sausage, that is, *carbohydrates, proteins, and fats.*

Changes in Composition During Fermentation

Carbohydrates. Organic acids, among which lactic acid may be the most important, are formed from the glycogen of the meat and from the sugars added during preparation. When the *Micrococcus* culture is suitable, the production of lactic acid increases in speed, and thus the pH value decreases more rapidly than without inoculation (Fig. 6).

In this way the conditions in sausage are changing fast so that the growth of, for example, bacteria proteolyzing meat is no more possible, as was shown previously (Fig. 3). As is known, the proteolytic bacteria are generally sensitive to the effect of NaCl and acidity (Pohja *et al.*, 1960; Pohja and Niinivaara, 1960).

Proteins. Because the proteins are undoubtedly of very great significance to the *flavor, texture, and color* of the product, we have tried to explain the changes of the proteins during the ripening. Some of the most important observations of this investigation are surveyed below.

To examine the changes in the structure of proteins, extract "A" was prepared by extracting the sample with cold water, and "B" by extracting it with trichloroacetic acid (Fig. 4). Examination of extract "A" showed that the *solubility* of proteins decreases during fermentation (Fig. 7). In this connection, no differences could be observed between inoculated and uninoculated sausages. This is also what one would expect because the employed micrococci cultures did not have any proteolytic characteristics. *Amino-nitrogen* content increases during fermentation from about 1% to 3% of total nitrogen (Fig. 8).

The changes in the individual *free amino acids* were examined by a paper chromatographic method that is half quantitative. The size of the spot was determined visually and recorded by a number on a certain scale. Regarding most amino acids there was an increase that, in general, appeared to be most intense during the first three days of fermentation (Fig. 9). Thereafter the changes were insignifi-

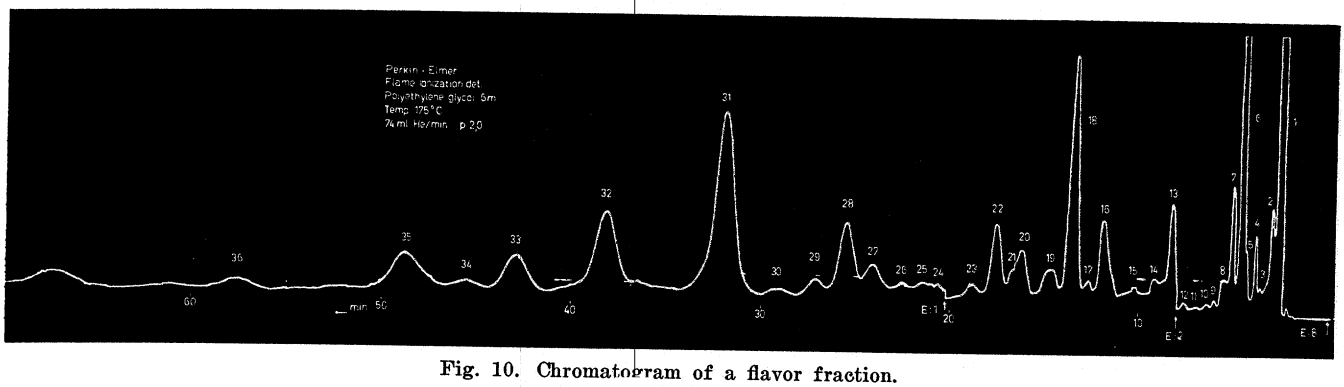

Fig. 10. Chromatogram of a flavor fraction.

how the amounts of compounds detected by gas chromatography and having a part in flavor production conditions are changed. It has not been possible to show changes of this kind by using the earlier methods when examining the changes in, e.g., compounds containing nitrogen.

When proceeding with these investigations we therefore hope to obtain results that will elucidate the part of bacteria in the production of the flavor of dry sausage.

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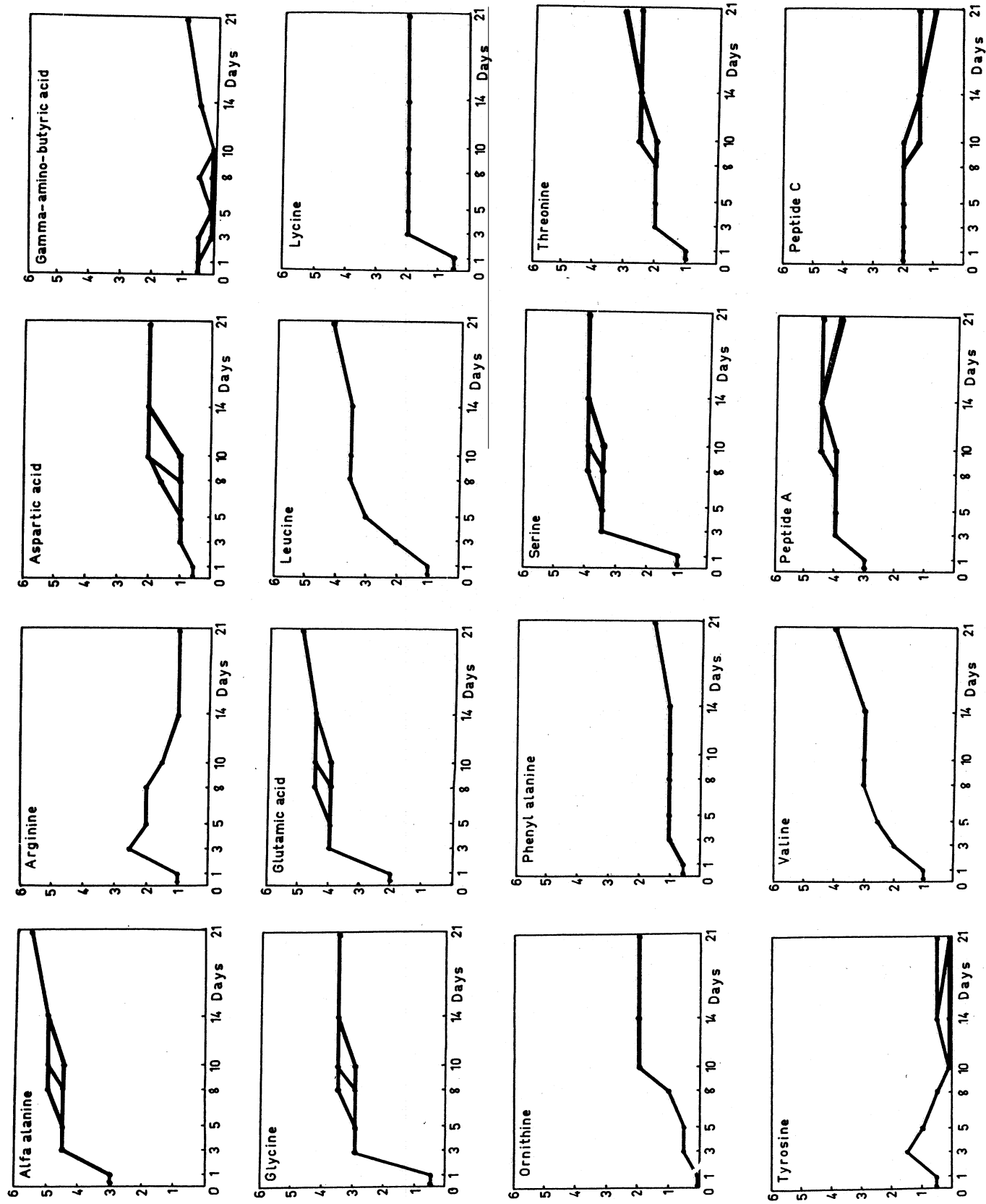

Fig. 9. Changes in free amino acids.